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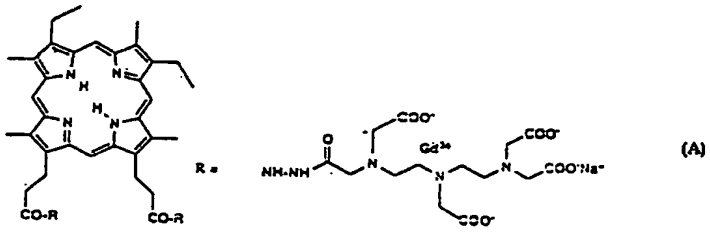

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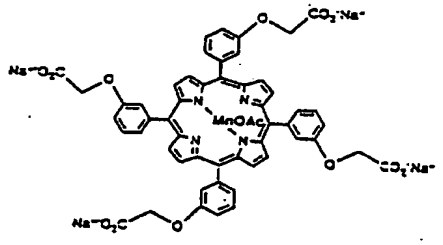

(B)

(57) Abstract

The invention relates to the use of porphyrin-complex or expanded porphyrin-complex compounds for the manufacture of a diagnosticum for the localization of an infarction and of a necrosis, wherein the infarction or necrosis may comprise an infarction of heart, kidney, intestine, lung, and/or brain, and wherein the porphyrin-complex compound may be Gd-MP and/or Mn-TPP. Gd-MP: Bis-Gd-DTPA-{Mesoporphyrin-IX-13, 17-bis[2-oxo-4,7,10,10-tetra-(carboxylatomethyl)-1,4,7,10-tetraazadecyl]-13, 17-diamide}, bis sodium salt of formula (A); Mn-TPP: Manganese-(III)-{Tetrakis-[3]-(carboxylatomethoxy-phenyl)-porphyrin}-acetate, tetra sodium salt of formula (B).

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The use of porphyrin-complex or expanded porphyrin-complex compounds as localisation diagnosticum for infarction or necrosis

The present invention relates to the use of porphyrin-complex and expanded porphyrin-complex compounds for use as a diagnosticum, in particular for use as a diagnosticum for the detection, localization, and monitoring of an infarction, and of a necrosis.

Suitable porphyrin-complex compounds are subject of DE-A-4,232,925, DE-A-4,305,523, EP-A-336,879, and EP-A-355,041. The subject matter of these applications are included by cross reference.

These porphyrin-complex compounds are used as a pharmaceutical preparation for the diagnosis and therapy of tumours.

Other suitable porphyrin-complex compounds are expanded prophyrin-complex compounds (17).

The present invention is based on the inside that these porphyrin-complex compounds can be used for the detection, localization, and monitoring of an infarction, and of a necrosis, such as ischemic, alcohol, and biliary obstruction, induced necrosis, and further laser induced hepatic, renal and muscular necrosis.

Hereafter the use as an infarction localization diagnosticum is primarily exemplified for a myocardial infarction and for a renal infarction, but it will be obvious for a skilled person that due to similar pathophysiological situations the same experimental findings apply to other infarction such as those of the intestines, lung, brain and the like.

Myocardial infarction is not a stable pathophysiological situation, but instead progresses to its definite form over several weeks to months. This process can be subdivided, although overlapping, in at least three periods. The first 24 hours after the start of ischemia

(acute evolving myocardial infarction) damage progresses as a wavefront phenomenon from the subendocardium to include the myocardium transmurally. During the second phase (established myocardial infarction) this area stabilizes and fibrosis is formed as a healing process. The third phase (healed infarction) starts after all the damaged tissue is replaced by a fibrotic scar. During this phase, considerable remodelling takes place. So far no accurate and reliable technique exists that can determine the evolution phase of the myocardial infarction antemortem.

The most important long-term prognostic factor after a myocardial infarction is the amount of myocardial tissue lost during this process. So far, no accurate and reliable technique exists to demonstrate the end-point, the amount of irreversibly damaged tissue antemortem.

In the three phases described above, it is of extreme importance to have an accurate status about the amount and localization of the affected myocardial tissue. During an evolving myocardial infarction, it is important to assess the amount of tissue at risk, the amount already lost, and from these parameters the amount of tissue that can be salvaged by reperfusion by thrombolysis or emergency surgical revascularisation, according to the hemodynamic status of the patient. In a patient with unstable angina, it is often impossible to discriminate between reversibly injured (akinetic, stunned) myocardium and irreversibly damaged tissue. This would nevertheless have a profound impact on the therapeutic strategy. In the case of complications in the phase of established infarction, requiring surgical intervention, it is known that mortality is highest when dead tissue is revascularized, causing hemorrhagic infarctions. An operative strategy of repair of the ventricular septum defect or mitral insufficiency with selective revascularisation of non-necrotic tissue could save lifes.

Up to now, a satisfactory in vivo method for localizing and defining an infarction and the size of an infarction has not yet been available, which impedes the progress of both the basic research and clinical practice (1). For instance, current imaging techniques such as echocardiography (2),

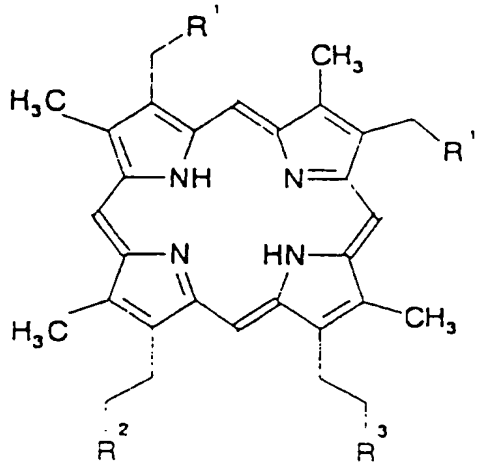
nuclear scintigraphy with perfusion and infarct avid tracers (3-5) and magnetic resonance imaging (MRI) without and with different contrast media (6-9) are still far from optimal in terms of sensitivity, specificity, spatial resolution, contrast and reliability (1).

Similar contemplations apply for infarctions of the kidney intestines, lung and brain.

Necrosis is a status of local tissue death, and results from the effects of diseases resulting in an adverse and detrimental effect on body tissue. Necrosis may be caused by radiation, injury, chemicals, local oxygen deficiency, infections, cancer, and the like. Monitoring, localization and detection of necrosis allowes the follow up and effectiveness determination of all kinds of diagnostic and therapeutic therapies and treatments.

The present invention relates to the use of these porphyrin-complex compounds or metalloporphyrins, for the localization, visualization of an infarction and of a necrosis. This invention is based on experimental results with myocardial and renal infarctions, and with hepatic, renal and muscle necrosis demonstrating an extraordinary effect with one-to-one correlation between magnetic resonance images (MRI) and histochemical preparations. This preclinical result open new horizons for especially the cardiac and necrotic imaging.

The porphyrin-complex compounds comprise a ligand having the general formula I



(I)

and at least one metal ion suitable for ex corporal determination. Suitable metal ions have an atomic number of 21-32, 37-39, 42-51 and 57-83.

In this general formula:

R^1 represents a hydrogen atom, a straight or branched C_1-C_6 alkyl group, a C_7-C_{12} aralkyl group or a OR' group wherein R' is a hydrogen atom or a C_1-C_3 alkyl group,

R^2 and R^3 represent a group CO-Z or a group $(NH)_o$-$(A)_q$-NH-D, wherein Z is a group OL with L is an inorganic or organic cation or a C_1-C_4 alkyl group, A is a phenylenoxy group, a C_2-C_{12} alkylene group possibly interrupted by one or more oxygen atoms, or a C_7-C_{12} aralkylene group, o and q independently represent an integer 0 or 1, and D represents an hydrogen atom or a group CO-A $(COOL)_o$-$(H)_m$ with m equals 0 or 1 under the provisio that the sum of m and o equals 1;

R^3 represents a group $(C=M)(NR^4)_o$-$(A)_q$-(NR^5)-K, wherein M represents an oxygen atom or two hydrogen atoms;

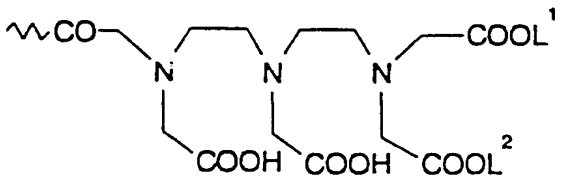
R^4 represents a group $(A)_q$-H; and

K represents a complex former having the general formula IIa or IIb, and

R^5 when K is formula IIa has the same meaning as R^4 and when K has the formula IIb has the same meaning as D, under the proviso that a direct oxygen-nitrogen bond is not allowed,

wherein L^1 has the meaning of a C_1-C_6 alkyl group or an inorganic or organic cation and wherein

L^2, L^3 and L^4 independently have the same meaning as L^1 or are an hydrogen atom, under the proviso that the complex former comprises at least two free carbon acid groups, and optionally for charge mutualization of the metalloporphyrin other anions, and pharmaceutically acceptable addition salts and carriers and diluants.



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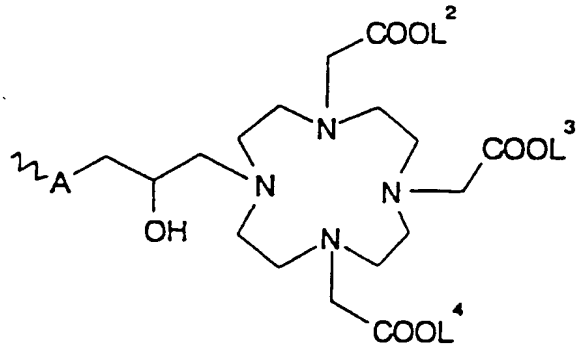


(IIb)

For MR localization the porphyrin-complex compounds comprises at least one paramagnetic metal ion, preferably di- or trivalent ions of the metal elements having the atomic number 21-29, 42, 44 and 57-70. Suitable metal ions are for instance chromium (III), manganese (II), manganese (III), iron (III), cobalt (II), cobalt (III), nickel (II), copper (II), praseodymium (II), neodymium (III), samarium (III) and ytterbium (III). Prefered are gadolinium (III), terbium (III), dysprosium (III), holmium (III), erbium (III) and iron (III).

For radioscintigraphic determination radioisotopes of the elements having the atomic number 27, 29-32, 37-39, 42-51, 62, 64, 70, 75, 77, 82 or 83 are preferred.

It is noted that when the complex compounds comprises various metal ions these metal ions may originate from the group for MR visualization and radioscintigraphic visualization.

Futhermore the metal ion may be complexed in the porphyrin skeleton, in the so called expanded porhyrin skeleton, and/or in the complex former.

Examples of the porphyrin-complex compounds are the disodium salt of the digadolinium complex of N,N'-Bis[9-

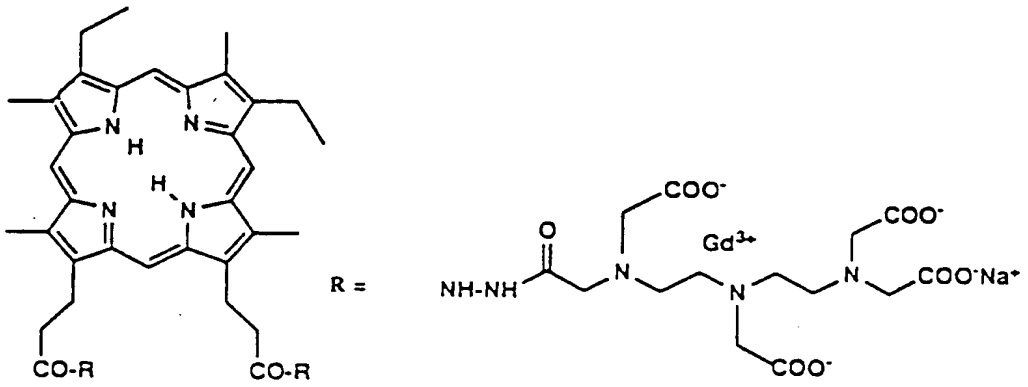
carboxylato-2,5,8-tris(carboxylatomethyl)-2,5,8-triazanonyl-carbamoyl]-mesoporphyrin-IX-13,17-diamides (Gd-MP).
The disodium salt of the digadolinium complex of manganese (III)- N,N'-Bis[11-carboxylato-2-oxo-4,7-bis(carboxylatomethyl)-10-(ethoxycarbonylmethyl)-1,4,7,10-tetraazaundecyl]-3,8-bis(1-propyl)-porphyrin-IX-13,17-diamides -acetates, and the digadolinium complex of manganese (III)- N,N'-Bis[11-carboxylato-2-oxo-4,7-bis(carboxylatomethyl)-10-(ethoxycarbonylmethyl)-1,4,7,10-tetraazaundecyl]-3,8-bis(1-propyl)-porphyrin-IX-13,17-diamides -acetates (Mn-TPP).

The diagnosticum has the form of a pharmaceutical formulation suitable for intra-veneous or intra-arterial injection in the form of a solution or suspension. The diagnosticum may comprise suitable additives, such as a buffer (tromethamine), complex formers such as diethylenetriaminpenta-acetic acid, electrolyte such as sodium chloride, antioxydantia such as ascorbinic acid.

Furthermore additives, tencides and the like may be added.

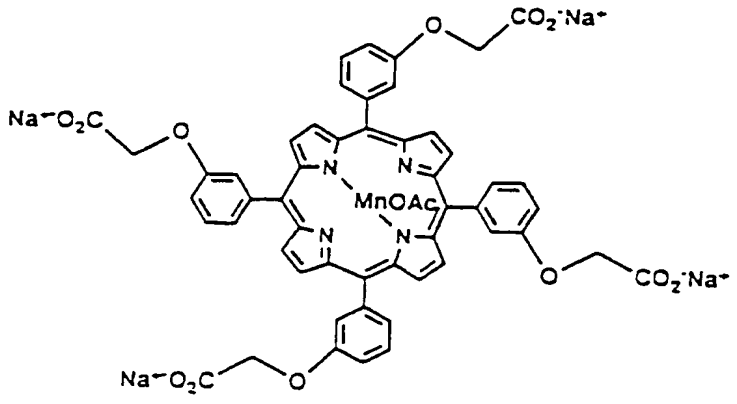
Gd-MP

Bis-Gd-DTPA-{Mesoporphyrin-IX-13, 17-bis[2-oxo-4, 7, 10, 10-tetra-(carboxylatomethyl)-1, 4, 7, 10-tetraazadecyl]-13, 17-diamide}, bis sodium salt



Mn-TPP:

Manganese-(III)-{Tetrakis-[3]-(carboxylatomethoxy-phenyl)-porphyrin}-acetate, tetra sodium salt



The diagnosticum may comprise the porphyrin or expanded porphyrin complex compound in an amount of 0.0001 - 10.0 mmol/kg body weight. Preferred is an amount of 0.005 - 2 mmol/kg body weight, more preferred 0.01 - 1.0 mmol/kg body weight. The actual dose is also dependent on the infarction to be localized, on the patient and on the localization technique to be used.

Hereafter the use of a diagnosticum comprising these specific metalloporphyrins, for the localization of an infarction and of necrosis, according to the invention will be shown for the visualization of an acute myocardial infarction and renal infarction, and of necrosis. The result obtained so far did not encounter neither false positive nor false negative findings. Striking is the almost perfect matching of the ex corporal localization and the histochemical confirmation.

In the experiments two paramagnetic metalloporphyrins have been used which were originally developped as potential tumour specific MRI contrast agents (10-14). Gadolinium mesoporphyrin (Gd-MP) and manganese tetraphenylporphyrin (Mn-TPP) have been used.

Example 1

The model of myocardial infarction was produced in rats by ligation of the left coronary artery according to an established technique (15). Two groups of rats (12 in each) with myocardial infarction aging 2 to 24 hours received intravenously either Gd-MP (IDF GmbH, Berlin) or Mn-TPP (IDF GmbH, Berlin) at doses of 0.1, 0.05 and 0.01 mmol/kg (4 rats each). After an interval of 3 to 24 hours postinjection, axial and coronal T1 weighted spin echo MR images were obtained immediately before and after sacrificing the animals. The excised heart was incubated with triphenyl tetrazolium chloride (TTC), which is a reliable histochemical staining to distinguish the infarcted from the non-infarcted myocardium (16). In addition, two groups of rats (6 in each) were used as controls and underwent the same imaging and histochemical procedures, i.e. one group with infarction but without contrast agent injection, the other group with

injection (3 with Gd-MP, 3 with Mn-TPP) but without infarction. The difference between the infarcted and non-infarcted myocardium seen on MR images was quantified by measuring the signal intensities (SI) with a monitor defined region of interest and expressed as contrast ration (CR):CR = SI infarct / SI noninfarct (mean ± SD). The metal content of the tissue was measured by ICP-AES. Finally the MR images were carefully compared with the corresponding macro- and microscopic tissue preparations and correlated with the results of local metal content measurement.

The infarct of the 6 control rats could not be discerned by MRI without contrast media. However, 3 to 24 hours after injection of either Gd-MP or Mn-TPP, all 24 rats with myocardial infarction exhibited on MR images a clear delineation of the infarcted areas of the heart, which precisely matched the areas of negative staining on the histochemical samples (Fig.1). The CRs between the infarcted and noninfarcted regions were 3.40 ± 0.26 at 3 hours and 1.92 ± 0.17 at 24 hours after contrast agent injection. Even the small dose of 0.01 mmol/kg worked well (CR = 1.84 ± 0.13 at 10 hours postinjection). Neither false positive findings (i.e. contrast enhancement in noninfarcted area) nor false negative findings (i.e. infarcted myocardium not enhanced with the agents) were obtained. The Gd content was as much as 9 fold higher in the infarcted myocardium (Table 1), suggesting that the MRI signal enhancement is mainly due to a preferential accumulation of metalloporphyrins in infarcted tissue.

Example 2

Using the same model of myocardial infarction, in two rats minor necrotic lesions were found at the ligation sides.

The MRI was performed 10 hours after Mn-TPP (0.05 mmol/kg body weight) intervenous injection. The technique is so sensitive that even lesions between 1 to 2 mm in size were easily detectable (Fig.2).

Example 3

A rat with partial renal infarction of the right kidney was injected with Gd-MP (0.1 mmol/kg body weight by intervenous injection).

24 Hours after Gd-MP injection, the Gd-content (measured with ICP-AES technique) of the infarcted and non infarcted kidney were similar but the signal intensities was at least two fold higher for the infarcted kidney (Table 2). Presumably the mechanism for metalloporphyrin induced specific enhancement seems not only related to an accumulation of the porphyrin-complex compound in the infarcted tissue. An increased relaxivity of the metalloporphyrins induced by a change in local molecular environment plays also a role in the observed increased signal intensity (Fig. 3).

Example 4

In order to evaluate the potential of these agents for the detection and monitoring of other types of necrosis following experiments were performed.

Spontaneous liver necrosis was induced by ligation of the common bile duct in rats. 72 Hours after surgery both types of metalloporphyrins (Gd-MP and Mn-TPP) were intravenously injected at a dose of 0.05 mmol/kg. Already 10 minutes after injection areas of strong enhancement could be observed in the liver. This enhancement lasted for about one week. Macroscopic examination confirmed that the enhancing areas corresponded to cholestatically related liver necrosis. A second experiment consisted in the induction of local necrosis in liver, kidney and muscle in rats by local injection of absolute alcohol. Imaging 8 to 24 hours after alcoholisation of both metalloporphyrins caused a concentric enhancement of the induced lesions. Those remained enhanced for several days. Macroscopy and microscopy after sacrifice confirmed the necrotic nature of the lesions (Fig. 4A, 4B, 4C).

Example 5

Infarcted myocardium induced the model of example 1 and laser induced necrosis using standard laser model techniques were studied in rats.

Before injection of the contrast agents, the induced necrosis were not visible on MR images. However, positive enhancement appeared in these lesions after contrast agent injection and persisted for more than 24 hours.

The contrast agents used were Mn-TPPS4 (Mn-meso-tetra-(4-sulfonato-phenyl)-porphyrine (available from Porphyrin Products Inc., Logan, Utah, USA), in an amount of 0.05 mmol/kg, and Gd-Mn-porphyrin (Mn(III)-{N-Bis-[11-carboxylato-2-oxo-4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazaundecyl]-methylpyrroporphyrin-XXI-amide}-acetate, Gd-complex, sodium salt can be prepared according to example 1/14 in WO84/07894).

Methylpyrroporphyrinethylester (Aldrich Chemicals) is reacted with hydrazine in pyridine and subsequently with manganese acetate in acetic acid. The obtained intermediate is reacted with DTPA-monoanhydride-monoethylester in absolute N,N-dimethylformamide and addition of triethylamine. After hydrolysis and neutralisation complexation is carried out with the use of gadolinium acetate in an amount of 0.05 mmol/kg.

The experimental results are summarized in tables 3 and 4.

The fact that necrosis of different origine, vascular and biliary infarction and alcoholisation, all show similar degrees of enhancement opens new prospectives for the monitoring of therapies that ultimately cause tissue necrosis, such as radiotherapy, chemotherapy, thermotherapy, laser therapy, ultrasound and radiofrequency ablation, alcoholisation, etc. ...

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Table 1

Gd content and MRI signal intensity in rats with myocardial infarction measured 24 hours after Gd-MP (0.05 mmol/kg)

Myocardium	Gd (µmol/g) ICP-AES	Signal Intensity (ROI)
infarcted	0.065 ± 0.006	422 ± 31
non infarcted	0.007 ± 0.002	193 ± 17
ratio*	9.29	2.19

Note: *infarcted/non infarcted

Table 2

Gd content and signal intensity in a rat with partial renal infarction measured 24 hours after Gd-MP (0.1 mmol/kg).

Tissue	Gd (µmol/g) ICP-AES	Signal intensity (24 h)
infarcted kidney	0.75	1340
non-infarcted kidney	0.79	638

Table 3

MRI Findings after myocardial infarction*

	Signal Intensity		CR
	Normal Myocardium	Infarcted Myocardium	Infarct/Normal
Mn-TPPS4	343	583	1.7
Gd-Mn-porphyrin	320	669	2.1

* The agents were injected 12 hours before MR imaging in rats with myocardial infarction (MI) aging 24 hours.

Table 4

MR Imaging in Laser Induced Necrosis*

	Signal Intensity			CR
	Precontrast	24 h post-contrast		
		Liver tissue	Necrosis	Lesion/ Normal
Mn-TPPS4	518 ± 21	625 ± 34	1063 ± 52	1.7
Gd-Mn-porphyrin	501 ± 30	593 ± 27	1126 ± 18	1.9

* The signal intensities of the liver and necrotic lesions were derived from pre- and 24 hours postcontrast MR images.

Legends for figures:

Fig. 1 (A-C). MRI and macroscopic photographs of a rodent heart with myocardial infarction. The MRI was performed 24 hours after Gd-MP (0.1mmol/kg) intravenous injection and immediately after sacrificing the animal.

A, B: Coronal (A) and axial (B) T1 weighted spin echo images (TR/TE = 300/15 msec, slice thickness = 2 mm, FOV = 100 mm, matrix size = 256 x 256, NEX = 6) display a strongly signal enhancment in almost all left ventrical wall including part of the ventricular septum (arrows) but not some papillary myocardial structures (arrowheads). The graduation near the frame on the right side represents 1 cm.

C: Axial section of the heart on a similar plane to the axial MR image (B), incubated with 1 % triphenyl tetrazolium chloride (TTC) for 15 minutes and fixed overnight with 10 % formalin, shows the left ventrical wall including part of the septum as unstained, (pale) infarcted area. Arrowheads indicate the intact myocardial papillae shown in B.

Fig. 2 (A-C). MRI and macroscopic photographs of a rodent heart with local injury caused by ligation. Such minute necrotic lesions were found at the ligation sites in two rats who failed to form real infarction and were excluded as successful models from the study. The MRI was performed 10 hours after Mn-TPP (0.05 mmol/kg) intravenous injection and immediately after sacrificing the animal.

A, B: On both the coronal (A) and axial (B) MR images (the same parameters as in fig. 1), an hyperintense lesion(arrow) of approximately 1 mm in size can be clearly seen in the left ventricular wall, despite a partial volume effect (i.e. the diameter of the lesion is smaller than the thickness of the MR slice; otherwise the lesion would appear brighter). The graduation near the frame on the right side represents 1 cm.

C.: TTC stained axial section of the heart on a similar plane to the MR image (B) displays the ligature and adjacent minute unstained necrotic lesion (arrow).

Fig. 3 (A - D). MRI and macroscopic photographs of a rat with partial renal infarction in the right kidney.

A - C: Axial T1 weighted spin echo images (TR/TE = 600/15 msec, the rest parameters are the same as in Fig. 1 A and B.

A : On precontrast plain scan in the right kidney, infarcted and noninfarcted parts cannot be discerned.

B : Ten minutes after Gd-MP 0.1 mmol/kg) intravenous injection, the noninfarcted parenchyma (lower part) is strongly enhanced in contrast with the unenhanced infarcted parenchyma (upper part), which is gradually filled up with time by the agent (images not shown).

C : Forty-eight hours postcontrast, when the signal intensity of the noninfarcted kidney (lower part) has almost normalized, the infarcted upper part of the kidney is still strikingly enhanced.

D : Macroscopic view of the right kidney on a similar section as in C. Note how well the areas of the infarcted and noninfarcted parenchyma seen on the specimen match with the contrast enhanced MR image (C).

Legends for Fig. 4 A-D: Axial T1 W SE MR images and macroscopic photographs of rat liver with alcohol induced coagulation necrosis.

A. On precontrast image, the 10 hours old necrotic lesion (arrow) is isointense and therefore can not be detected.

B. Ten minutes after intravenous injection of gadolinium mesoporphyrin (Gd-MP, 0.05 mmol/kg), the lesion (arrow) appears hypointense with some central bright spots (blood vessels). The lesion concentrically enhances with time whereas the liver intensity progressively decreases (images not shown).

C. 24 hours later when the liver intensity has largely decreased, the bright coagulation lesion appears bright (arrow) with some central dark spots. This suggests a specific retention and a strong affinity of the metalloporphyrin for the necrosis.

D. Macroscopic photograph of the liver section in the plane similar to MR images. The alcohol induced coagulation necrosis (arrow) has the same morphology with some intralesional blood vessels, as shown on the contrast enhanced MR images.

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P HP/sm/L-68pct

CLAIMS

1. The use of porphyrin-complex or expanded porphyrin-complex compounds for the manufacture of a diagnosticum for the localization of an infarction and of a necrosis.

2. Use of claim 1, wherein the infarction and/or the necrosis comprises an infarction or necrosis of hart, kidney, intestine, lung, and/or brain.

3. Use of claim 1 or 2, wherein the porphyrin-complex compounds comprise a radioactive and/or (super) paramagnetic label metal.

4. Use of claim 1-3, wherein the diagnosticum comprises the porphyrin-complex compound in an amount of 0.001 - 1.0 mmol/kg body weight.

5. Use of claim 1-4, wherein the porphyrin-complex compound is Gd-MP and/or Mn-TPP.

*****

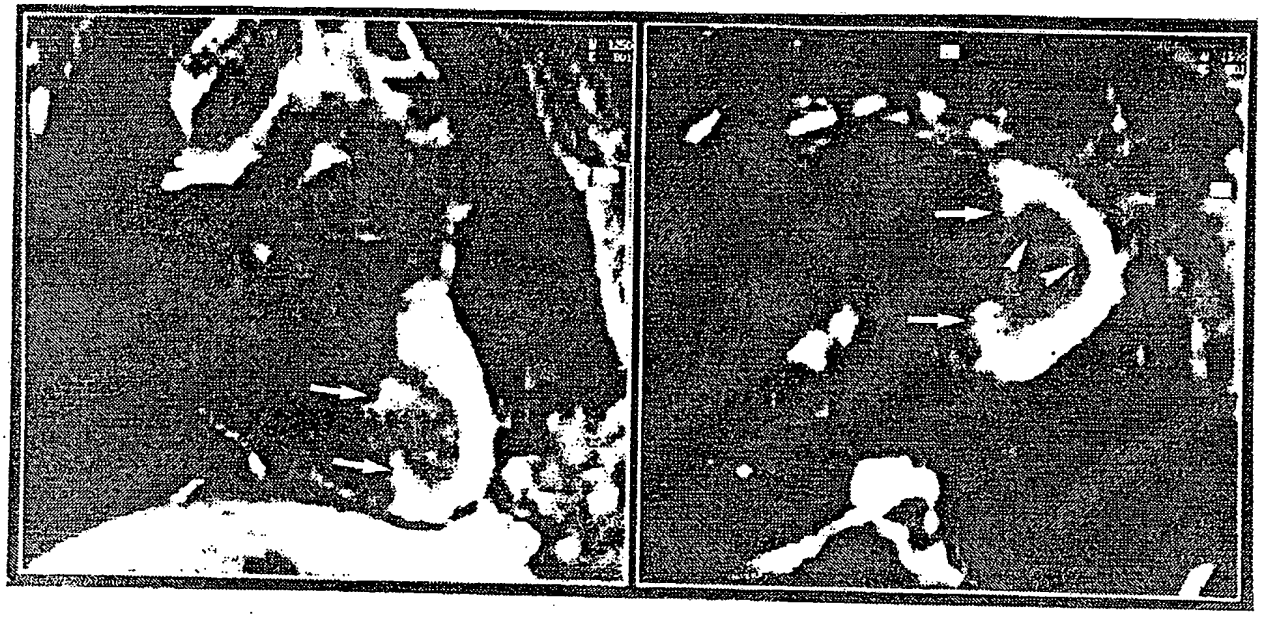


Fig. 1A

Fig. 1B

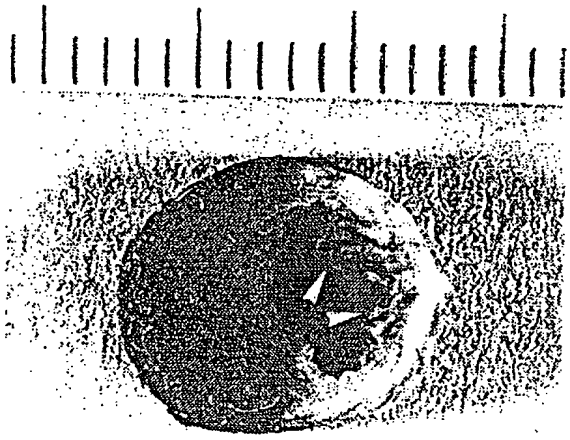


Fig. 1C

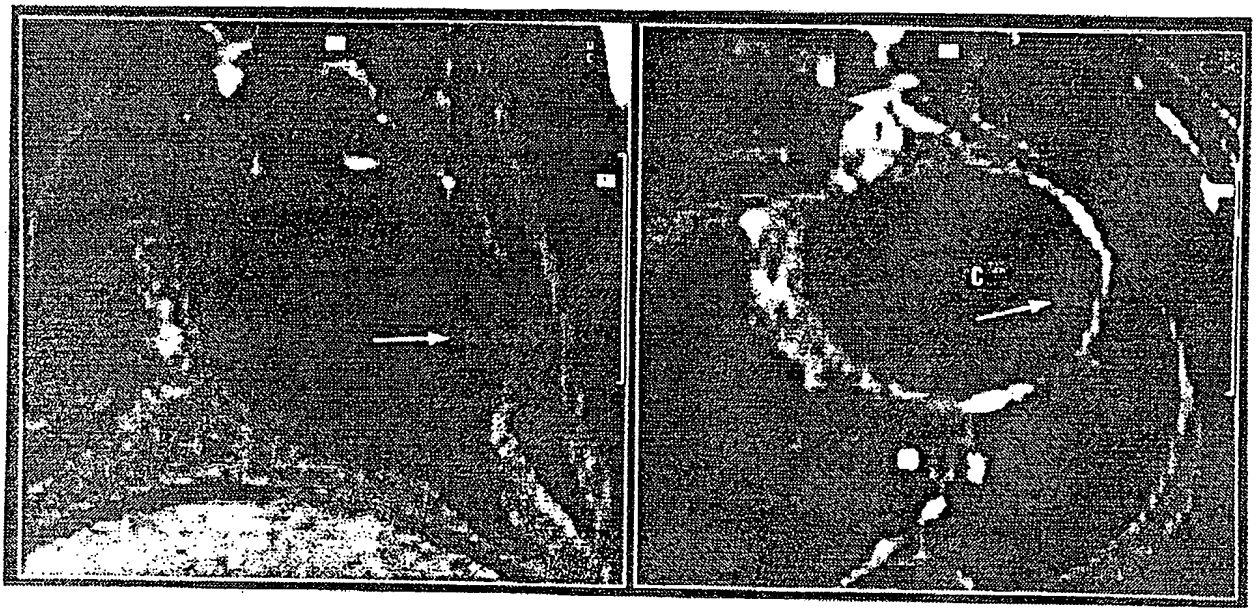


Fig. 2A

Fig. 2B

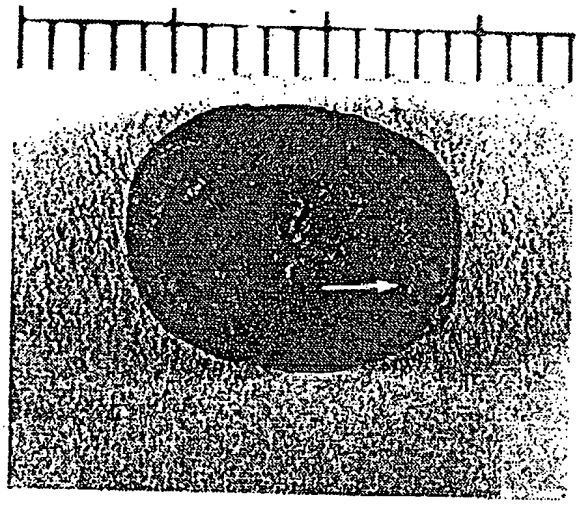


Fig. 2C

Fig. 3A

Fig. 3B

Fig. 3C

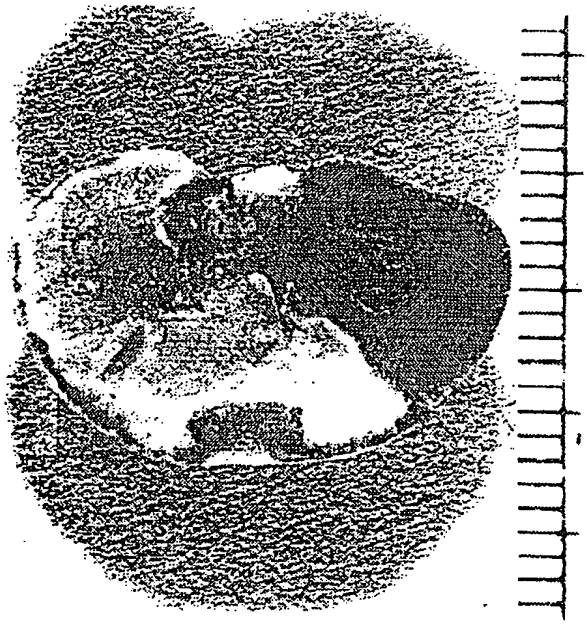
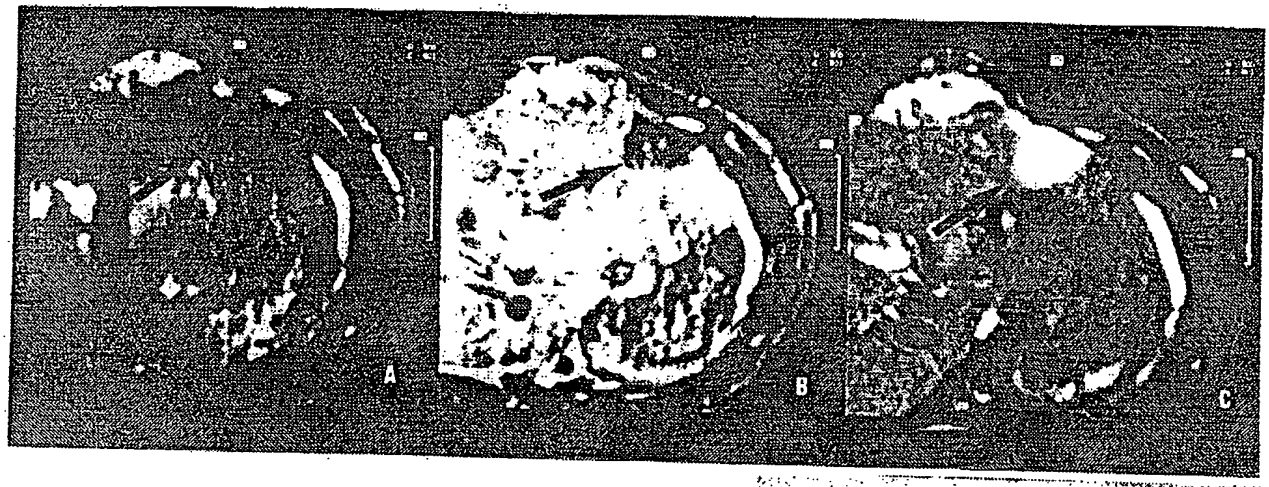


Fig. 3D

Fig. 4A Fig. 4B Fig. 4C



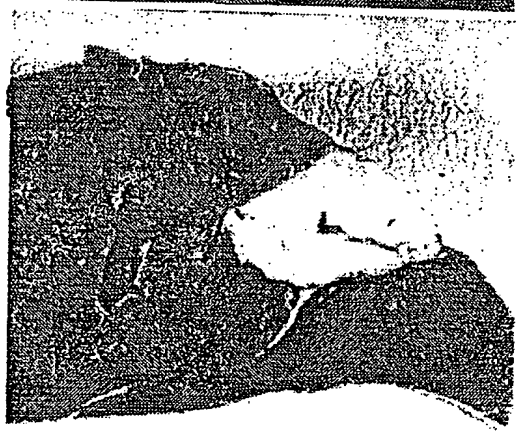


Fig. 4D

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/EP 95/01762

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K49/00 A61K51/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO-A-92 06097 (QUEEN'S UNIVERSITY AT KINGSTON) 16 April 1992 see page 18 - page 19 see examples ---	1-5
X	AM J PHYSIOL, VOL. 264, NO. 2 PT 2, PAGE(S) H294-301, February 1993 US, INCE C ET AL 'Heterogeneity of the hypoxic state in rat heart is determined at capillary level.' see abstract see page H295, right column see page H297, right column --- -/--	1-5

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 1995	06.10.95.

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Dullaart, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BIOLOGICHESKIE NAUKI., NO. 6, PAGE(S) 22-6, 1992., MAKSIMOV, GV. ET AL 'Mekhanizmy konformatsii porfirinov gemoglobina krovi v norme i pri patologii.' see abstract	1-5
Y	BIOCHEMISTRY, VOL. 16, NO. 11, PAGE(S) 2560-2565, 1977., ROSS P D ET AL 'MYOGLOBIN AS AN OXYGEN INDICATOR FOR MEASURING THE OXYGEN BINDING CHARACTERISTICS OF A MODIFIED MYOGLOBIN DERIVATIVE CONTAINING COVALENTLY BOUND MESO HEME.' see abstract see page 2560	1-5
Y	ITO, U. ET AL. (ED.). ACTA NEUROCHIRURGICA SUPPLEMENTUM, 60; 9TH INTERNATIONAL SYMPOSIUM ON BRAIN EDEMA, TOKYO, JAPAN, MAY 16-19, 1993. 1994, SPRINGER-VERLAG: VIENNA, AUSTRIA; NEW YORK, NEW YORK, USA. PAGE(S) 347-349., BOCKHORST K ET AL 'Localization of experimental brain tumors in MRI by gadolinium porphyrin.' see abstract; figures * Discussion *	1-5
Y	DE-A-42 32 925 (INSTITUT FÜR DIAGNOSTIKFORSCHUNG GMBH AN DER FREIEN UNIVERSITÄT BERLIN) 31 March 1994 cited in the application see examples	1-5
Y	DATABASE DISSERTATION ABSTRACTS UMI AAD93-12017, FAUSTINO, PATRICK JOHN 'EVALUATION OF METALLOPORPHYRINS AS CONTRAST AGENTS FOR TUMORS IN MAGNETIC RESONANCE IMAGING' see abstract & DISSERTATION ABSTRACTS INTERNATIONAL. VOLUME: 54/01, SECTION: B, PAGE: 208. ABSTRACT OF THESIS (PH.D.), 1992, THE AMERICAN UNIVERSITY, 265 PAGES,	1-5

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE DISSERTATION ABSTRACTS UMI AAI9239269, HEMMI, GREGORY, WILLIAM. 'LANTHANIDE COMPLEXES OF THE TEXAPHYRIN CLASS OF EXPANDED PORPHYRINS.' see abstract & DISSERTATION ABSTRACTS INTERNATIONAL. VOLUME: 53, NUMBER: 08, SECTION: B, PAGE: 4110. ABSTRACT OF: THESIS (PH.D.)--THE UNIVERSITY OF TEXAS AT AUSTIN, 1992, 239P.,	1-3
X	DATABASE DISSERTATION ABSTRACTS UMI AAI9323498, MODY, TARAK, DHIRAJ. 'THE DESIGN, SYNTHESIS, AND STRUCTURAL CHARACTERIZATION OF SCHIFF-BASE "EXPANDED PORPHYRINS" (TEXAPHYRIN, MRI CONTRAST AGENTS).' see abstract & DISSERTATION ABSTRACTS INTERNATIONAL. VOLUME: 54, NUMBER: 04, SECTION: B, PAGE: 1958. ABSTRACT OF: THESIS (PH.D.)--THE UNIVERSITY OF TEXAS AT AUSTIN, 1993, 312P.,	1-3
Y	J NUCL MED, JUL 1985, VOL. 26, NO. 7, PAGE(S) 756-60, FOSTER N ET AL 'Delineation of a transplanted malignant melanoma with indium-111-labeled porphyrin.' see abstract see page 758, right column - page 759, left column	1-5
Y	MAGN RESON MED, 1987, VOL. 4, NO. 1, PAGE(S) 24-33., LYON R C ET AL 'TISSUE DISTRIBUTION AND STABILITY OF METALLOPORPHYRIN MRI CONTRAST AGENTS' see abstract see "Discussion"	1-5
Y	EP-A-0 336 879 (SCHERING AG) 11 October 1989 see examples see claims	1-5
P,X	WO-A-94 19352 (DIAGNOSTIKFORSCHUNG INST FREIEN UNIV BERLIN) 1 September 1994 see examples 2,4,6,12B,13A see examples 14-16	1-5

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INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/EP 95/01762

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X, L	CIRCULATION, OCTOBER 1994, VOL. 90, NO. 4, PART 2, PAGE(S) I468, ABSTRACT NO. 2512, NI Y ET AL 'Metalloporphyrin enhanced magnetic resonance imaging of acute myocardial infarction' see abstract L: Different structure of Gd-MP and Mn-TPP	1-5

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. ..al Application No
PCT/EP 95/01762

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9206097	16-04-92	AU-B-	655942	19-01-95
		AU-A-	8612291	28-04-92
		CA-A-	2093361	06-04-92
		EP-A-	0553116	04-08-93
		NZ-A-	240057	26-07-94
		US-A-	5219878	15-06-93
DE-A-4232925	31-03-94	WO-A-	9407894	14-04-94
		EP-A-	0662972	19-07-95
		NO-A-	951166	27-03-95
EP-A-0336879	11-10-89	DE-A-	3809671	28-09-89
		AU-B-	3148589	21-09-89
		JP-A-	1275583	06-11-89
		PT-B-	90033	31-05-94
		US-A-	5284647	08-02-94
WO-A-9419352	01-09-94	DE-A-	4305523	18-08-94

INTERNATIONAL SEARCH REPORT

I. national application No.
PCT/EP 95/01762

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although the claims mentioned below are directed to a method of treatment of the human or animal body (Rule 39.1(iv)PCT), the search has been carried out based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims searched incompletely: 1-5

SEE ANNEX!

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP95/01762

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

REASON: In view of the large number of compounds, which are defined by the general formula or definition used in the claims, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application (see PCT search guidelines, Chapter III, paragraph 3.6).